R126 Subc4 B5 Cancel

54. (Added) An influenza antigen for an animal species comprising a fusion product, said fusion product comprising

(i) an extracellular part of an M2 membrane protein of an influenza A virus of said animal species, or of a similar integral membrane protein of an influenza B or C virus of said animal species; and

(ii) a presenting carrier.

REMARKS

Applicants have amended claims 26-28, 31, 32, 38-40 and 46, and cancelled claim 33 without prejudice. Applicants have also added claims 47-49. None of these amendments introduce any new matter (*infra*). As such, claims 26-32, 34-41 and 46-49 are pending in this application. Applicants respectfully request reconsideration of the application in view of these claim amendments and the following discussions.[1]

I. Summary of the Invention

Influenza viruses (types A, B, and C) contain two highly immunogenic but very variable proteins on the membrane – hemagglutin (HA) and neuraminidase ("N protein"). Currently available flu vaccines are all derived from these two proteins. Due to the high mutation rates of these proteins, these vaccines confer protection against only the same strain of virus from which the vaccines are developed. Whenever mutations occur on the proteins, the vaccines will lose efficacy against the mutant virus. The mutant virus can then spread rapidly, causing the next epidemic. To prevent new flu

1 Rejections directed to cancelled claim 33 are moot and therefore not discussed.

epidemics, the flu vaccines have to be changed almost every year to follow the antigenic drift of the virus.

This invention solves this problem by providing vaccines derived from amino acid sequences that are highly conserved in epidemic influenza strains within the same animal species (e..g, human). In influenza A virus, such sequences are found in membrane protein M2. In influenza B and C viruses, such conserved sequences are found in the NB protein and the CM2 protein, respectively. Applicants believe that individuals vaccinated with these vaccines will become immune to all strains of influenza virus of the same type (i.e., type A, B, or C). Thus, these new vaccines can potentially eradicate flu epidemics.

II. Claim Amendments

Claim 26 has been amended to particularly point out that the recited integral membrane protein is from a "human" influenza virus. Support for this amendment appears throughout the specification, e.g., at p. 3. This claim is also amended to recite an extracellular part of an integral membrane protein of a human influenza B or C virus that is similar to the M2 protein of influenza A virus. Support for this amendment appears in the first "wherein" clause in unamended claim 26 itself. Per the Examiner's request, the reference to Table 1 in this claim has been replaced with SEQ ID NOs. Claim 26 is also amended to remove language with which the Examiner has raised issues.

Claim 27 is amended to recite "peptide or polypeptide" to clarify the meaning of "(poly)peptide." This amendment is purely grammatical in nature. The same amendment is made to claim 28.

Claim 28 is further amended to recite the Markush group in a more formal manner. Also, "one or more C3d domains" is replaced with "C3d, polypeptides comprising multiple copies of C3d." Support for this amendment appears in the specification at p. 7, lines 34-37.

Claims 31 and 32 are amended to remove "additional domain."

Claim 38 is amended to replace "membrane fragment" with "lipid bilayer or cell wall." This amendment clarifies the meaning of "membrane fragment." Support for the amendment is inherent in the original term "membrane," since all bio-membranes are either lipid bilayers or cell walls. See also the specification at p. 49, lines 26-29.

Claim 39 is amended to replace "cell envelope" of the bacteria *Lactococci* cells with the more customary term "cell wall." Support for this amendment appears at p. 23, line 10 and p. 49, lines 26-29 of the specification.

Claim 40 is amended to improve the form of the claim.

Claim 46 is amended to remove the dependency on an unelected claim. Support for this amendment appears in unelected claims 42 and 44.

Claim 47 is added. This claim depends from claim 26 and specifies that the influenza antigen comprises a cytokine. Support for this claim appears in the specification at p. 46, line 26 to p. 47, line 8, and at p. 51, line 35 to p. 52, line 25.

Claim 48 is added. This claim depends from claim 26 and specifies that the influenza antigen comprises a vaccine adjuvant that is not Freud's adjuvant. Support for the new claims appears in the specification at p. 6, lines 9-13.

Claim 49 is added, which is directed to an influenza antigen for an animal species. Support for this claim appears in the specification at p. 9, lines 24-27, and

throughout the working examples.

III. Objections of Claims

Claims 27, 28 and 46 stand objected to for informalities. Office Action, p. 2. Specifically, the Examiner states that there is inconsistency between claims 27 and 28 in terms of which portion of "polypeptide" is put in parentheses, and that in any case, there is no need to use the parentheses.

Applicants acknowledge the inconsistency and apologize for the typographical error. The parentheses should have flanked "poly" in claim 28, as in claim 27. The term "(poly)peptide" is intended to mean "a peptide or a polypeptide." Both "peptide" and "polypeptide" are polymers of amino acid residues linked by peptide bonds. But customarily, peptides refer to low molecular weight polymers while polypeptides encompass larger polymers.[2] Both peptides and polypeptides can be used as presenting carriers in the claimed influenza antigens. To recite this claim element in a more formal manner, applicants have replaced "(poly)peptide" with "a peptide or polypeptide" in claims 27 and 28.

Claim 46 is objected to for depending from nonelected claim 44. Amendments to this claim obviate this objection by removing the reference to claim 44.

IV. Rejections Under 35 U.S.C. § 112, ¶ 2

Claims 26-32, 34-41 and 46 stand rejected as being indefinite. Office Action, pp. 3-5. Applicants respectfully traverse these rejections in view of the claim amendments set forth above.

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2 See, e.g., Dorland's Illustrated Medical Dictionary (cited in the Office Action at p. 4), where a peptide is defined as "any member of a class of compounds of low molecular weight that yield two or more amino acids on hydrolysis" (emphasis added).

I

The Examiner contends that claim 26 is indefinite for several reasons. The Examiner asserts that the terms "functional fragment," "modified versions," "statistically significant immunoprotection," and "immunoprotective dose" are vague. The amendments made to this claim render these issues moot, obviating the need to address the merits of these rejections.[3]

The Examiner also requests that SEQ ID NOs be used in lieu of reference to Table 1. Applicants have amended the claim accordingly.

The Examiner further contends that "similar integral membrane protein of influenza B or C virus" is unclear because applicants have not defined what those proteins are. Applicants respectfully traverse.

It is true that there is no protein in influenza B or C virus that is called "M2." However, a person of ordinary skill in the influenza virology art would have known that the NB protein in influenza B virus and the CM2 protein in influenza C virus are the functional as well as structural equivalents of M2.

Applicants first discuss the NB protein. M2 and NB are the **only** type III integral membrane proteins expressed by influenza A and B viruses, respectively. In Fields,[4] it is stated at p. 1365, first col.: "The influenza virus NB glycoprotein has the same uncommon membrane orientation {type III integral membrane protein (380)} as the influenza virus M2 protein encoded by RNA segment 7 (see below and Fig. 3)." Fig. 3

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3 Applicants point out that amended claim 26 continues to encompass functional variants (e.g., fragments or modified versions) of SEQ ID NOs:1, 2, and 3.

4 This book is cited by the Examiner in the *Notice of References Cited.*

(top of p. 1359) is a schematic representation of M2 and NB, showing the structural equivalence. Indeed, the N-terminal extracellular domains, the hydrophobic transmembrane domains and the intracellular domains of both proteins are of very similar lengths.[5] Further, both proteins form tetramers. And the intracellular domains of both proteins are fatty acylated via a thioester linkage.

M2 and NB are also functionally similar. Both proteins exist sparingly on the respective viruses but are much more abundant on infected cells.[6] Both proteins form ion channels in the cell membrane.[7]

Similarly, the prior art has shown that CM2 in influenza C virus is structurally and functionally similar to M2 in influenza A virus.[8]

In sum, a skilled artisan would have readily understood that "a similar integral protein of influenza B or C virus" refers to the NB and CM2 proteins of the respective viruses.

II

The Examiner rejects claim 28 for reciting the phrase "one or more C3d domains" and for reciting an improper Markush group. This rejection should be withdrawn in light of the claim amendment.

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5 William and Lamb, *Mol Cell Bio* 6(12):4317-28 (1986). Exhibit 1. Brassard et al., *Virology* 220(2):350-60 (1996). Exhibit 2.

6 Betakova et al., *J Gen Virol* 77 (Pt11):2689-94 (1996). Exhibit 3. See also Brassard, *supra.*

7 Hay, AJ (1998) Functional Properties of the Virus Ion Channels, Chapter 7, pp. 74-81, in "*Textbook of Influenza*," Nicholson KG, Webster RG & Hay AJ, editors, Blackwell Science. Exhibit 4.

8 Hongo et al., *J Virol* 71(4):2786-92 (1997). Exhibit 5. Pekosz and Lamb, *Virol* 237(2):439-51 (1997). Exhibit 6.

III

Claim 30 stands rejected for reciting "peptide mimetics." The Examiner states that the nature of mimicry is not defined and that there is "inconsistency between a component that mimics a peptide in claim 30 and a peptide mimic that is non-peptidic in structure from claim 29." Applicants respectfully disagree.

Peptides and polypeptides consist of L-amino acids linked by peptide bonds. A peptide mimetic contains other building blocks and/or other types of chemical linkage, but binds to the same ligand as the peptide (or polypeptide) that it mimics.[9] Thus, a peptide mimetic and the peptide or polypeptide it mimics possess different chemical compositions and structures. There is no inconsistency between claims 29 and 30. Peptide mimetics of claim 29 can be those that mimic the peptidic carriers recited in claim 28.

IV

Claim 31 is rejected for reciting "additional domain." Applicants have amended claim 31 and dependent claim 32 to render the rejection moot.

V

With regard to claim 38, the Examiner states that it is unclear whether the term "membrane fragment" refers to the membrane of an influenza virus or to the membrane of the acceptor cell, and that if the latter is the case, the term conflicts with claim 37, because an acceptor cell membrane to which the fusion protein is anchored would not comprise membrane fragments, but an intact membrane.

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9 Moore, *Trends Pharmacol Sci* 15(4):124-9 (1994). Exhibit 7. Ripka and Rich, *Curr Opin Chem Biol* 2(4):441-52 (Aug 1998). Exhibit 8.

Applicants respectfully submit that the term refers to any bio-membranes (e.g., viral membrane, cellular membrane or cell walls). Since all bio-membranes are lipid bilayers or cell walls, applicants have replaced the term with "a lipid bilayer or cell wall" to clarify the meaning of the claim. Support for this amendment is discussed above.

VI

Claim 39 stands rejected for reciting "cell envelope." The Examiner points out that cells do not have envelopes. The amendment made to this claim obviates the rejection.

VII

Claim 40 stands rejected for reciting "for example." Applicants have replaced that term with "optionally."

VIII

Claim 46, a product-by-process claim, stands rejected for lacking description of how the process makes the claimed product unique. Applicants have amended the claim, specifying that the claimed influenza antigen comprises the same fusion product as recited in amended claim 26. The instant rejection can be withdrawn.

V. Rejections Under 35 U.S.C. § 112, ¶ 1

Claims 26-32 and 34-41 stand rejected for lack of enablement. Office Action, pp. 5-10. Applicants respectfully traverse in view of the claim amendments.

I

The Examiner first contends that the specification teaches only the N-terminal portion of the M2 protein, and does not teach any other functional fragments of the M2 protein. Office Action, p. 6, first full ¶. Amendments made to claim 26 have

rendered this rejection moot. Thus, applicants need not address the merit of this rejection.

II

The Examiner further asserts that the specification does not adequately teach which extracellular fragments of the M2 protein would be immunoprotective. Office Action, p. 8, lines 3-4. In a similar vein, the Examiner asserts that the specification also does not enable all possible amino acid sequence derivatives of the M2 extracellular domain (i.e., modified versions) that could react with polyclonal antiserum "since polyclonal antibodies are not immunospecific." Office Action, p. 6, second full ¶.

Applicants respectfully disagree. First of all, Table 1 of the specification provides three different sequences of the entire M2 extracellular domain (i.e., SEQ ID NOs:1, 2, and 3). Any person of ordinary skill in the molecular biology art could make mutated versions (e.g., deletional mutants such as fragments, insertional mutants, and mutants with amino acid substitutions) of these sequences using routine recombinant techniques. For instance, he/she could use primers, as those illustrated in Figs. 1-3, 6, 10, 13, 14, 17-25, and 29, to introduce mutations into the cognate M2 extracellular domain by polymerase chain reaction (PCR). See, e.g., the specification at pp. 23-24. Once the mutants are made, he/she could readily identify those with immunoprotective characteristics by following the guidance set forth in, e.g., pp. 32-39 of the specification. For instance, the mutants can be screened with polyclonal anti-M2 antibodies described in the specification.

The Examiner asserts that polyclonal antibodies are not immunospecific, citing Estabrook for support. Applicants strongly disagree. Polyclonal antibodies can be

as specific as monoclonals. Long before the discovery of monoclonal antibodies in 1975 by G. Köhler and C. Milstein, polyclonal antibodies were routinely used. Even today, polyclonal antibodies are still widely used for research, diagnosis, and therapy. Estabrook does not contradict this fact. It merely says: "monoclonal antibodies are more precise reagents than conventional antisera . . ." (emphasis added). This statement by no means amounts to a suggestion that polyclonal antibodies are not precise enough for use in applicants' invention.[10]

In sum, armed with applicants' disclosure, it would be a routine matter for a person of ordinary skill in the art to modify the M2 extracellular domain and screen for those modified versions (e.g., fragments and substitutional mutants) that are immunoprotective. Routine screening is not undue experimentation. *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). Indeed, neither the statutes nor the case law mandates that an applicant disclose and test every single species of a claimed genus in his/her disclosure.

III

The Examiner then asserts that the specification does not describe "how one would obtain every possible domain . . . that would enhance cellular imunogenicity of" the claimed influenza antigen. Office Action, p. 7, first ¶. This rejection is erroneous.

As discussed above, a specification need not disclose every single species in a claimed genus. Indeed, MPEP § 2164.03 states:

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10 In fact, polyclonal antibodies are more useful than monoclonal antibodies in certain circumstances. Monoclonal antibodies are highly precise because they recognize a single epitope. But because a protein presents to the immune system a collection of epitopes, polyclonal antibodies raised against a specific protein can be much more sensitive than a monoclonal antibody in detecting this protein.

> For a claimed genus, representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if one skilled in the art (in view of level of skill, state of the art and the information in the specification) would expect the claimed genus could be used in that manner without undue experimentation. Proof of enablement will be required for other members of the claimed genus only where adequate reasons are advanced by the examiner to establish that a person skilled in the art could not use the genus as a whole without undue experimentation. (emphasis added)

In the case at hand, applicants did disclose "representative examples" of additional domains, such as C3d, T cell epitopes (*infra*) and cytokines (e.g., p. 51, line 35 to p. 52, line 6). Applicants also provide "a statement applicable to the genus as a whole" – that the additional domains can enhance the cellular immunogenicity of the claimed influenza antigen. Yet, the Examiner merely conclusorily states that the specification does not teach every possible additional domain, without advancing any reasons why applicants' teachings are not sufficient. This is not enough. MPEP § 2164.03, *supra*.

The Examiner further states that the specification does not teach how a skilled artisan could immediately identify every influenza-specific T helper or cytotoxic T cell epitope. Again, applicants disagree.

T cell epitopes have been studied extensively in immunology, and there are standard methods to identify them.[11] In particular, numerous influenza T cell epitopes have been identified both in the mouse and human systems. These epitopes are present in nearly all influenza proteins.[12] Indeed, there is at least one T cell helper epitope in the M2 extracellular domain that is functional at least in Balb/c mice. Its effect is illustrated in

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11 See, e.g., J. E. Coligan et al., Eds., *Current Protocols in Immunology*, 1994, Jossey-Bass Company (ISBN 0-471-30660-6); and C. A. Janeway et al., Eds., *Immunology*, 4th Ed., Elsevier Science/Garland Publishing, 1999.

12 Gao et al., *J Immunol* 147:3268-3273 (1991). Exhibit 9. Levi et al., *Vaccine* 13:1353-1359 (1995). Exhibit 10. Uger et al., *J Immunol* 160:1598-1605 (1998). Exhibit 11.

Fig. 9 of the specification (post-challenge sera e). It can be seen that in mice that have been primed, i.e., treated with an M2 vaccine, the titers of sera e were much higher than in the unprimed control group, despite the fact that all mice received an equal dose of challenge virus. The M2 extracellular domain T cell epitope clearly helped the primed mice to mount a more vigorous antibody response upon the challenge with the live virus, compared to the unprimed control mice.

Thus, given the guidance in the specification, a skilled artisan would have known to use the large number of known influenza T cell epitopes to enhance the cellular immunogenicity of the claimed fusion protein.

IV

The Examiner further alleges that the specification does not teach what "would be considered a similar M2 integral membrane protein from influenza virus B or C since these viruses do not contain M2." Office Action, p. 7, penultimate line to p. 8, line 2.

As discussed above, a skilled person in the virology art would know what similar M2 integral membrane proteins from influenza viruses B and C refer to – the NB protein in influenza virus B and the CM2 protein in influenza virus C. Further, the amino acid sequences of the extracellular domains of both NB and CM2 are known. See, e.g., Williams (*supra*; Exhibit 1), at p. 4318, Fig. 1, for the extracellular domain sequence of NB (amino acid residues 1-18); and Pekosz (*supra*; Exhibit 6), at p. 440, Fig. 1B, for the extracellular domain sequence of CM2 (amino acid residues 25-47). Thus, a skilled person in the art could readily make influenza B and C vaccines based on the respective viruses' extracellular domains, as applicants have exemplified for the M2 protein in the

working examples.

V

The Examiner next asserts that a skilled artisan "would doubt that the claimed fusion protein would be able to prevent and treat any influenza virus infection [because the] working examples fail to demonstrate that the mice were protected against every type of influenza virus . . . ." (Office Action, p. 8). The Examiner cites Fields, Zebedee and Slepushkin for support. Fields allegedly teaches that influenza viruses undergo antigenic shift and antigenic drift. Zebedee allegedly teaches that the N-terminus of the M2 protein is not conserved, especially at residues 11 and 14, and that M2-specific monoclonal antibodies could not prevent initial infection. Slepushkin allegedly teaches that M2 protein vaccination shortened the duration of virus shedding but the immune response in humans with the M2 protein after infection would be of "lower activity and/or less durable." The Examiner further alleges that the data from the working examples were inconclusive. Applicants traverse each of these points below, in view of the amendments made to claim 26, which now recites **human** influenza viruses.

1. The Prior Art

Fields correctly recognizes that influenza viruses undergo antigenic drift and antigenic shift which affect the HA and N proteins. In fact, two of the present inventors (Willy Min Jou and Walter Fiers) were the first to document these drift and shift phenomena in molecular terms.[13] It is precisely because of these phenomena that so far no universal influenza vaccine has been developed (*supra*).

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13 Verhoeyen et al., *Nature* 286(5775):771-6 (1980). Exhibit 12. Fang et al., *Cell* 25(2):315-23 (1981). Exhibit 13.

Applicants discovered that, unlike HA and the N protein, M2 does not undergo antigenic drift and shift, and is highly conservative among influenza strains **within** a host species.[14] In fact, the M2 extracellular domain of human influenza A virus has remained essentially the same since 1933, when the first viral isolate was obtained, despite the occurrence of two major pandemics in the intervening period. See, e.g., Table 1 of the specification.

The Examiner asserts that M2 is not conserved and cites Zebedee for support. Zebedee describes the sequence variation of M2 between **human** and **avian** strains of influenza A virus. The differences at residues 11 and 14 cited by the Examiner are between human and avian strains. See, e.g., the Abstract; p. 2765, right col., last ¶; Fig. 4; and the paragraph bridging the left and right columns at p. 2770. Zebedee, however, demonstrates that **human** strains of influenza A virus are highly conserved.[15] See, e.g., Fig. 4, where the M2 amino acid sequences of five human strains – A/Udorn/72, A/WSN/33, A/PR/8/34, A/Sing/1/57, and A/HK/8/68 – are listed. The M2 extracellular domains of all five strains are absolutely conserved (identical to SEQ ID NO:1 of the present application), except for A/PR/8/34 and A/Sing/1/57, where residue 21 is G instead of D (SEQ ID NO:2 of the present application).

Notably, applicants show that vaccines based on both SEQ ID NOs:1 (e.g., IM2HBcm) and 2 (e.g., IPM2HBcm) had the same efficacy in mice against subsequent challenge with influenza virus 30 HAU X-47, whose M2 protein contains SEQ ID NO:1

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14 Thus, new claim 49, directed to animal influenza virus antigens derived from an influenza virus of the same animal species, is also patentable.

15 So is true for influenza virus within another animal species.

in its extracellular portion.[16] Specification, p. 43, line 3 to p. 45, line 17. This result further demonstrates that the new vaccine can protect against both homologous and heterologous infections.[17]

In sum, applicants made the significant discovery that vaccines based on the highly conserved extracellular domain of the influenza A virus M2 protein of a given animal species (e.g., human) are protective against any strain of influenza A virus of this animal species. Zebedee does not cut against this significance.

The Examiner also contends that Zebedee teaches that an anti-M2 monoclonal antibody ("mAb") cannot prevent initial viral infection, although they restrict viral growth, and as a result, the skilled artisan would doubt that the vaccines of this invention would be immunoprotective. Applicants disagree.

First, applicants point out that Zebedee's results were obtained ***in vitro*** with a single **mAb** (14C2). A skilled artisan simply would not know whether these results could be extrapolated to ***in vivo*** systems, where vaccination typically elicits **polyclonal**, rather than monoclonal, antibodies. Indeed, vaccination induces immunological processes that are far more complex than those observed in tissue culture systems.

Second, contrary to the Examiner's argument, the prevention of initial infection is not necessarily always the goal of a vaccine. Very often, an initial infection is necessary for a vaccine to effect its immunoprotectiveness. In this context, it is noted that

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16 Baez et al., *J Infect Dis* 141:362-365 (1980).

17 Homologous infection refers to infection by the same strain of virus from which the vaccine is derived. Heterologous infection refers to infection by a strain of virus different from which the vaccine is derived.

influenza neuraminidase-induced antibodies cannot prevent an initial infection. Nonetheless, neuraminidase-based vaccines have been shown to be effective in humans.[18] Thus, the fact that Zebedee's mAb cannot prevent initial infection is not dispositive as to whether the vaccines of this invention are efficacious in humans.

The Examiner also cites Slepushkin for presenting data that would allegedly cast doubt on the operability of the claimed invention.[19] Applicants traverse. The procedure described in this reference can hardly be considered as a basis for development of an influenza vaccine and is fundamentally different from applicants' invention.

First, the M2 preparation used by Slepushkin to immunize mice was a crude cellular fraction. It was an enriched cellular membrane preparation in which the M2 protein represented a small fraction of total protein. This means not only that such an undefined preparation cannot be used as a vaccine for any clinical purposes, but also that the contaminants derived from the insect host cells and the baculovirus might have affected the outcome of the experiment.

Second, the nature of the protective activity is undefined as Slepushkin. In fact, the authors of Slepushkin admits that they could not transfer immunoprotective activity by using sera from their M2-vaccinated mice (p. 1402, left col., first ¶). In contrast, applicants unequivocally showed that sera from mice vaccinated with a M2 extracellular fragment conferred passive immunity. See the specification at p. 37, bottom ¶, entitled "Passive immunization"; and Fig. 8 and the paragraph bridging pp. 53-54,

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18 Kilbourne et al., *Vaccine* 13(18):1799-803 (1995). Exhibit 14.

19 This reference is discussed in the present specification at p. 5, last ¶.

where the results of passive immunization were presented and discussed. Thus, the protection seen in Slepushkin was clearly different from the protection seen in applicants' systems.

Given these apparent differences between Slepushkin and this invention, a skilled artisan would not infer from Slepuskin's teachings that the present invention is not operable within the full scope of the claims.

2. Data of Applicants' Working Examples

The Examiner contends that data from applicants' working examples are inconclusive with regard to the protective nature of the antigen. Office Action, pp. 9-10. Applicants address each of the Examiner concerns below.

First, the Examiner is concerned with applicants' statement that the vaccinated mice demonstrated high morbidity upon subsequent viral infection. Specification, p. 55, lines 29-30. The Examiner is correct that for the human population at large, a vaccine is preferred to also prevent morbidity. In humans, morbidity caused by flu consists mainly of coughing, fever and myalgia. These symptoms are not observed in mice, and in any event, are difficult to quantify. Therefore, it is a common practice to evaluate vaccines in mice on the basis of protection against a potentially lethal challenge, as this provides a clear cut result: life or death. Specification, p. 55, lines 25-36. But in order to obtain lethality in placebo-treated mice, a very high dose of viral challenge has to be used. In fact, Slepushkin, a reference cited by the Examiner herself, uses a similar method to test the immunoprotective efficacy of its M2 preparation. See p. 1400, right col., last ¶, entitled "Protective effect of recombinant Bac-M2 vaccine against **lethal challenge** with homologous or heterologous influenza virus" (emphasis added).

In the experiment referred to on p. 55 by the Examiner, a 5 LD_{50} dose was administered. This means that the dose used was five-fold higher than the dose required to kill 50% of the challenged mice. Under such severe conditions, it is not surprising that the mice, even vaccinated and therefore surviving the viral infection, showed morbidity such as drop in body temperature or loss of weight.[20] But the fact that the vaccinated mice had a much higher survival rate than the control mice is more than sufficient to demonstrate that the vaccines of this invention are highly effective. Specification, p. 46, lines 4-17; and p. 53, line 35 to p. 54, line 53.

The Examiner next alleges inconsistency in the experimental data presented at pp. 53 and 54 and Fig. 28.[21] Specifically, the Examiner points out that all of the mice that were passively immunized with IM2HBcm antisera were protected against viral challenge, but the mice that only received IM2HBcm antigen died at the same rate as the negative control group; and that protection observed in one experiment and lack of protection in another experiment with the same M2 extracellular region demonstrates unpredictability.

The Examiner is mistaken. At pp. 53 and 54, the specification states in regard to passive immunization:

> The survival is shown in figure 28. In both control groups only <u>one mouse out of 12 survived</u> the lethal influenza challenge, while <u>11 out of 12</u> mice immunized with 3 x 10 pg [sic; should read μg] IM2HBcm or all passively

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20 It is possible to evaluate less stringent parameters under conditions of non-lethal challenge. For example, one can challenge the immunized and control mice with a ten-fold lower concentration of virus, viz. 0.5 LD_{50}. This concentration is still considerably more than the amount of virus that causes natural infection in humans.

21 The Office Action points to Fig. 32. But there is no Fig. 32 in this application. It seems that the Examiner is referring to Fig. 28, which appears on sheet 32 of the submitted drawings and is discussed in the paragraph bridging pp. 53 and 54.

> immunized mice were protected. This experiment demonstrates that anti-M2 antibodies produced during the vaccination account for the observed protection. (emphasis added)

Thus, contrary to the Examiner's assertion, the mice that only received IM2HBcm antigen (filled squares in Fig. 28) died at a much lower rate (1/12) than the negative control mice (filled diamonds in Fig. 28) (11/12). In sum, these data demonstrate that immunization with an M2 extracellular domain-based vaccine consistently provides immunoprotection and this protection is humoral because it can be transferred by passive immunization through serum.

The Examiner further contends that a skilled artisan would doubt applicants' data on passive immunization because passive immunization is known to be brief in the host. The Examiner cites Cruse for support.

Applicants do not dispute that passive immunization is temporary and cannot provide long term protection, and is hence not a substitute for vaccination. However, applicants' experiment with passive immunization was merely used to demonstrate that the vaccine described in the application induced protective antibodies in the serum. Indeed, applicants' claims do not relate to passive immunization. Thus, the Examiner's concern can be laid to rest.[22]

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22 For completeness of record, applications point out that passive immunization provide protection for weeks or even months, if not years, sufficient in some cases to limit or even cure an ongoing infection. It can thus be life-saving, for example, in immuno-depressed patients or when no vaccine is available (e.g., in the case of Ebola infection). According to the *Compendium of Medicines*, immunoglobulins intended for passive treatment of infections are available against Hepatitis B virus, Tetanus, Rabies, Cytomegalovirus, Botulism, Gangrene, etc. The monoclonal antibody Synagis® (palivizumab), developed and sold by Medimmune Inc. (Gainthersburg, MD; http://www.medimmune.com), is becoming widely used to combat serious lower respiratory tract disease caused by respiratory syncytial virus (RSV) in pediatric patients. By the same token, antibodies against the M2 extracellular domain are not substitutes for vaccines but can be of great use for treatment of an immuno-depressed flu patient.

VI. Rejection Under 35 U.S.C. § 102(b)

Claim 46 stands rejected as being anticipated by Melnick. Applicants have amended the claim to specify that the claimed influenza antigen comprises a fusion product that is derived from the extracellular domain of an influenza virus. Melnick does not teach such an influenza antigen.

VII. Certified Copy of Priority Document

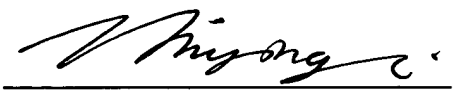
Applicants acknowledge the requirement under 35 U.S.C. § 119(b) to file a certified copy of prior application EP 97202434.3. Applicants will meet this requirement after the Examiner indicates allowable subject matter.

CONCLUSION

Applicants submit that the claims as amended are in condition for allowance. Applicants request early, favorable action from the Examiner.

To expedite prosecution, the Examiner is invited to telephone applicants' undersigned agent to discuss any issues that can be resolved over the telephone.

Respectfully submitted,



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Lillian Garcia

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APPENDIX OF CLAIM AMENDMENTS

26. (Amended) An influenza antigen comprising a fusion product, said fusion product comprising

(i) an extracellular part of an [influenza] M2 membrane protein **of a human influenza A virus, or of a similar integral membrane protein of a human influenza B or C virus** [or a functional fragment thereof or modified versions thereof,] and

(ii) a presenting carrier,

wherein said extracellular part **of the M2 membrane protein** contains all or part of **SEQ ID NO:1, 2 or 3** [the 23 amino acid extracellular domain (amino acid residues 2 to 24 as shown in Table 1) of an M2 protein of influenza A virus or of a similar integral membrane protein of influenza B or C virus, and

wherein said functional fragment is a fragment of an M2 protein capable of eliciting a statistically significant higher immunoprotection when administered in an immunoprotective dose to test members of a species, as compared to test members of said species not receiving the functional fragment, and

wherein said modified versions comprise one to three amino acid changes but still react with a polyclonal antiserum derived from immunized animals].

27. (Amended) The influenza antigen of claim 26, wherein the presenting carrier is a [(poly)peptide] **peptide or polypeptide**.

28. (Amended) The influenza antigen of claim 27, wherein the

presenting [poly(peptide)] **peptide or polypeptide** is selected from **the group consisting of** a hepatitis B core protein, [one or more C3d domains] **C3d, polypeptides comprising multiple copies of C3d**, tetanus toxin fragment C and yeast Ty particles.

31. (Amended) The influenza antigen of claim 26, [further comprising an additional domain for enhancing] **wherein the presenting carrier enhances** the cellular immune response immunogenicity of the antigen.

32. (Amended) The influenza antigen of claim 31, wherein [the additional domain is] **the presenting carrier comprises** an epitope of an influenza-specific T helper cell or cytotoxic T cell.

38. (Amended) The vaccine of claim 35, wherein the fusion product is part of a [membrane fragment] **lipid bilayer or cell wall**.

39. (Amended) The vaccine of claim 35, wherein the influenza antigen comprises *Lactococci* cells expressing the fusion product in or on their cell [envelope] **wall**.

40. (Amended) The vaccine of claim 35, further comprising one or more other influenza antigens, [for example] **optionally** selected from **the group consisting of** hemagglutinin, neuraminidase, nucleoprotein and native M2.

46. (Amended) An influenza antigen obtained by the [method of claim 44] **following steps:**

providing a nucleic acid construct that encodes a fusion product, said fusion product comprising (i) an extracellular part of an M2 membrane protein of a human influenza A virus, or of a similar integral membrane protein of a human influenza B or C virus, and (ii) a presenting carrier, wherein said extracellular part of the M2 membrane protein contains all or part of SEQ ID NO:1, 2 or 3;

introducing the nucleic acid construct into an acceptor cell;

culturing the acceptor cell under conditions that allow expression of the fusion product; and

optionally isolating the fusion product from the acceptor cell or its culture medium,

thereby obtaining an influenza antigen comprising the fusion product.